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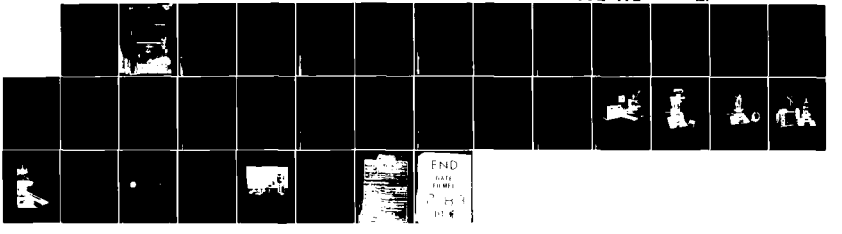

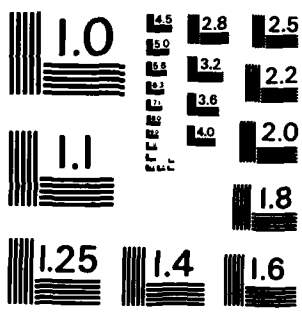

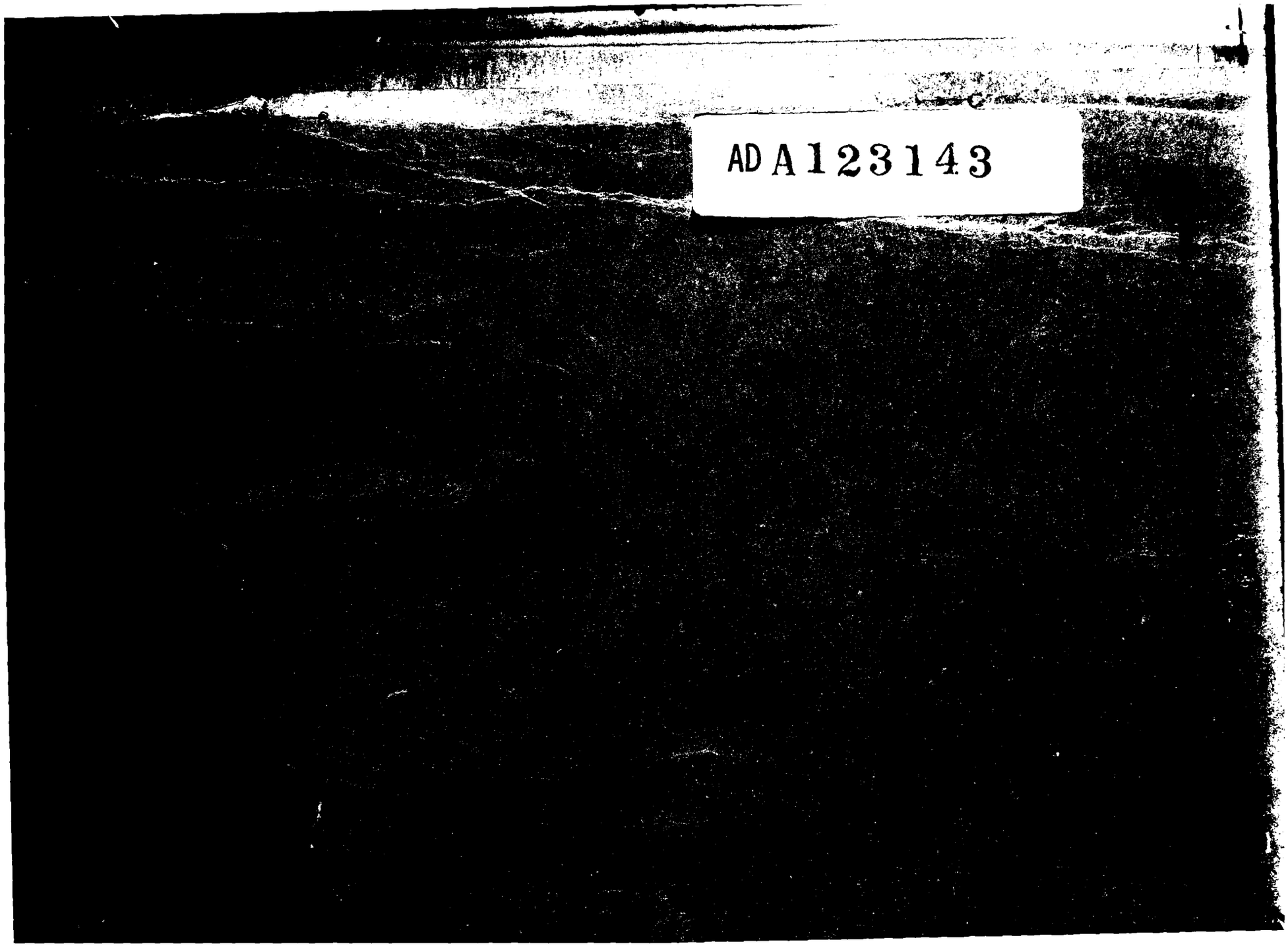
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SOME TECHNIQUES IN ANALYTICAL OPTICAL MICROSCOPY

by

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SUMMARY

A useful range of optical microscopical facilities has been built up in Analytical and General Chemistry Section, Materials Department.

The equipment and the techniques for which it can be used are described. Examples of applications are given.

Detailed procedures for some techniques are given in the Appendices.

LIST OF CONTENTS

TM Mat 397

1 INTRODUCTION

Facilities for analytical optical microscopy have been built up in Analytical and General Chemistry Section, Materials Department and provide a useful addition to the range of techniques employed. In qualitative analysis, elements can be identified by X-ray fluorescence spectrometry, emission spectrography or chemical tests and molecular features by infra-red spectroscopy and mass spectrometry. However, the crucial part of the analysis of unknown samples can often be the preliminary examination. For solid samples, optical microscopy is often the best approach to the preliminary examination because of the morphological details revealed: alternatively, microscopy can be useful in confirming the presence of particular compounds suspected from results of other techniques. When only a small amount of sample is available, microscopical techniques use only very little. They can usually be carried out on a single particle. Complete identifications are sometimes possible by microscopical methods alone.

The microscopical facilities described later are built around a Vickers M41 'Photoplan' microscope (Fig 1) fitted for transmitted and incident light, polarised light, dispersion staining and camera optics. Accessories include hot stages, micrometric graticules and a separate travelling microscope for density determinations.

This Memorandum describes some microscopical techniques which can be applied to chemical analysis at RAE. An elementary knowledge of microscope optics is assumed and descriptions can be found in any general book on microscopy, *eg* Ref 1 and the introductions to Refs 2 to 4.

2 EXAMINATION WITH THE POLARISING MICROSCOPE

2.1 Ordinary light

As a general rule, examination is carried out first at low magnification to give an overall impression of the sample: it is easy to 'lose sight of the wood for the trees' by adopting too high a magnification straight away. Once a general picture of the sample form has been gained, magnification can be increased as needed to study particular details more closely.

The information which can be got from simple examination in ordinary light includes morphology (*eg* size, geometry, colour and fracture characteristics) and refractive indices of isotropic substances. The use of the Particle Atlas[2] can often lead to partial or complete identification of unknowns with little more than this kind of examination.

Morphological analysis depends largely on experience and often on intuition. Particles as small as 2 μm in diameter can be described morphologically from examination at high magnifications, but electron microscopy would have to be resorted to for smaller particles. To overcome the subjective elements of morphological description, attempts are being made elsewhere to computerise classification systems based on the binary code[2]. A few examples of easily recognisable morphological characteristics can, however, be given. Biological material is usually easily recognisable from its 'organised' form. Natural fibres and hairs are easily distinguished (*eg* the 'twisted ribbon' appearance

of cotton and the scaly surface of wool are unmistakable) but synthetics are more difficult. Glassy or opaque spheres in a sample usually indicate a high-temperature history. Metal flakes often show tool marks.

The manipulation of single particles requires some practice. A portable laminar-flow clean bench is available to exclude dust particles from operations of this kind; the microscope itself can be mounted in the clean bench if necessary. Single particles are best picked up with a fine tungsten needle, made by etching a point on 0.25mm diameter tungsten wire with red-hot sodium nitrite. Encapsulation of particles in a thin film of collodion often makes them easier to handle. Fine micropipettes and membrane filters can aid in the handling of suspensions. An excellent description of the techniques for handling small particles has been given by Teetsov[5]. Descriptions of other methods of handling small amounts of material include those by Benedetti-Pichler[6], Schaeffer[3] and Burrells[4].

2.2 Polarised light[2,3,4,6,7]

The sample can be illuminated with plane-polarised light by inserting the polariser between the lamp-house and the condenser. The microscope can then be used to measure the refractive indices of anisotropic substances (see section 6) and to observe *pleochroism* and *twinkling*. Pleochroism is the change in colour of certain substances as they are rotated in plane-polarised light; twinkling is a change in brightness on rotation when the substance has two widely differing refractive indices. *Dispersion staining* (see section 7) can also be carried out in plane-polarised light to distinguish the different refractive indices.

2.3 Crossed polars

If, with the polariser still in position, the analyser is inserted between the objective and the eye-piece, a dark background is produced because light polarised in the plane of the polariser cannot pass through the analyser, the plane of which is usually kept at right angles to that of the polariser. Substances placed upon the stage of the microscope in this condition may appear dark whatever the sample's orientation, or bright with varied colours.

Those substances which remain dark are optically *isotropic, ie* their refractive indices are the same in all directions. Isotropic substances are either amorphous or belong to the cubic system of crystals.

The substances which appear bright with varying colours are optically *anisotropic*. These may be *uniaxial, ie* having two refractive indices, ε and ω, and belonging to either the tetragonal or hexagonal crystal systems, or *biaxial, ie* having three refractive indices, α, β and γ, and belonging to the orthorhombic, monoclinic or triclinic crystal systems.

Although anisotropic crystals appear bright at most orientations, there are always four positions, each at 90 deg to the next, in which they appear dark. These are termed the *extinction positions* and occur when the crystal's vibration direction corresponds with that of one of the polars so that no light can be transmitted. The angle observed

between an extinction position and the long axis of the crystal is called the *extinction angle*; this is sometimes a useful characteristic for aiding identification of an unknown and is easily measured using the rotating stage.

The colours seen when an anisotropic sample is observed between crossed polars are interference colours. The light which has passed through the polariser is polarised in one plane and when it subsequently passes through an anisotropic sample it is resolved into slow and fast rays at right angles to each other. As these rays travel through the sample at different characteristic velocities, they emerge at the top with a path difference, the *retardation*. When the two rays are recombined by the analyser, the phase difference caused by the retardation leads to destructive interference for certain wavelengths, with other wavelengths remaining. The combination of the remaining wavelengths gives rise to the interference colours observed through the eye-piece. The colours vary with the retardation and with the thickness of the sample and occur in the characteristic Newton's series. The series is divided into orders, each ending with a red-violet colour equivalent to one full wavelength retardation. The relation of colour, retardation and thickness is shown in the Michel-Lévy chart[2,9] and from this, if any two of the factors are known, the other can be found.

Additivity of retardances is made use of in *compensators*. If two anisotropic substances are superimposed, their retardations are added or subtracted according to their fast and slow directions. If the slow directions coincide, the retardations are added and a colour of a higher order to that given by either substance alone results; this will be the sum of the retardations on the Michel-Lévy chart. If, however, the slow directions are perpendicular, the retardations are subtracted, the colour is of a lower order and the result is the difference between the retardations. Compensators are plates of anisotropic crystals of carefully controlled thickness which can be slid into the microscope tube between the objective and the analyser at 45 deg to the directions of the polars. They are used to detect feebly-firefringent substances so that these may be differentiated from isotropic substances, and to pick out the fast and slow directions of crystals or fibres. A substance elongated in the direction of its high refractive index is said to have a positive *sign of elongation* but if elongated in the direction of its low refractive index, negative. *First-order red* (quartz), *quarter wave* (mica) and *wedge* compensators are available.

3 PHOTOMICROGRAPHY

It is useful in particle identification work to have a collection of photographs of known substances. The Particle Atlas[2] provides a good basic reference set but records of samples encountered in RAE work have also been used to build up a valuable collection.

In order to provide photographic records resembling as closely as possible what is actually seen in the microscope, colour transparencies are normally used. Mounted in a slide viewer, these give a very good likeness. Kodak Ektachrome 160 tungsten light film has been found to be suitable and can be processed in RAE Printing Branch.

The camera pillar is mounted in place of the monocular head (Fig 2) and includes the camera body, shutter and viewing eye-piece. The photocell of a simple exposure meter

is also fitted; the required exposure time is calculated from a factor found empirically from test exposures. For photographing samples between crossed polars, the exposure is determined by inserting the quarter-wave compensator[8].

4 MICROMETRY

Depth or thickness measurements can be made with the calibrated fine focus control from the difference in readings with the top and bottom of the sample in focus.

Sizes of specimen features are measured with eye-piece graticules previously calibrated with a stage micrometer. The conversion factors for the eye-piece graticule readings depend on the power of the eye-piece and the objective used. The smallest dimension measurable without introducing very large errors is about 0.5 μm.

A mechanical vernier stage is also available for larger measurements and a separate travelling microscope can be used horizontally or vertically.

5 HOT STAGE MICROSCOPY

5.1 Kofler hot stage[3,23]

The Kofler hot stage (Fig 3) provides the facility for observing the behaviour, in transmitted light, of materials heated to temperatures of up to 350 deg.

The sample is mounted between a half slide and a cover glass. Heating is controlled manually by a rheostat. Substances which show sharp melting points by the usual capillary tube method may betray the presence of traces of impurities by showing a melting range in the microscopical method.

The Kofler hot stage is also useful in the single variation method for the accurate determination of refractive index (see section 6).

5.2 High-temperature hot stage

The Stanton Redcroft HSM-5 high-temperature hot stage unit[24] (Fig 4), allows the observation of samples, in reflected light, at temperatures of up to 1000 deg. Oblique top illumination from an independent microscope lamp has been found to give a more satisfactory image than the incident light unit built in to the microscope. The temperature is controlled by a programmer with a choice of ten heating rates and the atmosphere in which the sample is heated can be controlled. Evolved gas analysis is possible if a flowing gas is used. The evolution of aldehydes from heated polymeric materials has been followed by passing the effluent from the hot stage through two drops of saturated *o*-anisidine solution and visually observing any colour change. It is hoped to be able to collect effluent in a tube packed with porous polymer for subsequent desorption into gas chromatographic equipment. Aluminium, inconel or platinum dishes are used to contain the sample; in the case of platinum, the weight loss of the sample during heating can be determined by weighing before and after on an electronic microbalance.

The high-temperature hot stage is particularly useful in the preliminary examination of unknown samples.

6 REFRACTIVE INDEX MEASUREMENTS

If an isotropic solid is immersed in a liquid of the same refractive index, light can pass straight through both and the solid is invisible. This is the basis of determining refractive indices of small particles under the microscope.

The refractive index of an isotropic substance is determined in ordinary light. As refractive index varies with light wavelength, a source of monochromatic light is best, and is conveniently supplied by mounting a wedge interference filter (Fig 5) beneath the condenser and setting it to a specific wavelength, usually 589 nm, that of the sodium D-line. Liquids of controlled refractive indices of between 1.400 and 1.700 at intervals of 0.002 are available. The refractive index of a liquid varies with temperature but the variation for a solid is negligible. The label on each bottle of liquid gives a temperature correction factor which should be applied when the ambient temperature is not 25 deg.

The technique involves finding the liquid in which the sample becomes invisible. To aid 'homing in' on the correct refractive index an effect called the *Becke line* is used. This is a bright halo around the image of the sample which moves towards the material (liquid or solid) of higher refractive index when the focus of the microscope is raised slightly, and towards the material of lower refractive index when the focus is lowered slightly. In this way one can always tell whether the immersion liquid is higher or lower in refractive index than the unknown solid. Repeated Becke line tests in various liquids lead to the liquid in which the sample is invisible. The accuracy of this simple method is the refractive index interval of the liquids available, *ie* 0.002.

More accurate determinations have been made by the *single variation method.* As mentioned above, the refractive index of a liquid varies significantly with temperature whereas that of a solid does not. A typical immersion liquid changes by about 0.0004 in refractive index per degree temperature change, so if the preparation of solid sample in liquid is mounted on the Kofler hot stage, precise control of the liquid's refractive index is possible. A liquid whose refractive index is greater than that of the sample but close enough to become equal to it below about 50 deg is used for the preparation and heated slowly until the sample 'disappears'. Refractive index measurements accurate in the third decimal place can be made in monochromatic light.

The maximum and minimum refractive indices of anisotropic solids can be found by alignment in polarised light, but is time consuming, especially for the fragmented crystals which usually occur in contamination samples.

7 DISPERSION STAINING

Dispersion staining[2,10-14] is an identification technique for transparent particles based upon the difference in *dispersion of refractive index* between the particle and the liquid in which it is immersed. This dispersion is usually greater for the liquid than for the solid (Fig 6) so that at a certain wavelength λ_0, light will pass through solid and liquid without deviation. Light of any other wavelength will be deviated.

The deviated light can be separated from light of wavelength λ_0 by means of a stop in the back focal plane of the objective (Fig 7). The stop may be central or annular. The annular stop gives an image coloured λ_0 and the central stop gives an image coloured a mixture of the deviated wavelengths, *ie* the complementary colour to λ_0. The annular stop gives a light background and the central stop a dark background. With either stop, the particle is invisible in monochromatic light of wavelength λ_0. If measurements of λ_0 are made for a series of liquids close in refractive index, a dispersion staining curve can be plotted which is characteristic of the solid sample (Fig 8).

If the sample is isotropic, only one dispersion staining colour is found whatever the orientation. Particles of a uniaxial substance all show a common colour at one pair of extinction positions but biaxial substances do not. However, if a large number of the biaxial particles are observed it is sometimes possible to find 'maximum' and 'minimum' colours corresponding to γ and α. β cannot be found.

Collections of dispersion staining data[2,15,16] can be used for identifications of unknowns.

Dispersion staining is also used for distinguishing particles of a particular substance from others present.

8 MEASUREMENT OF DENSITY

It is possible to determine the density of single particles by using a density gradient column of appropriate size. The column is set up in a melting point capillary tube using di-iodomethane and xylene to give a density gradient from 3.3 to 0.9. For each density determination, a column is calibrated with particles of substances of known density and their positions in relation to that of the unknown are measured with a travelling microscope (Fig 9). Suitable standard substances are shown in Table 1, and typical calibrations in Fig 10. The determination is accurate to the first decimal place only.

A mixture of particles of different densities can be separated on the density gradient column described and, with practice, it is possible to collect the separate fractions from it onto a membrance filter for further examination.

9 APPLICATIONS AT RAE

All the techniques described have been used in numerous analytical jobs at RAE. According to the requirement, they are used individually, in combination with each other or in combination with non-microscopical techniques such as infra-red spectroscopy and pyrolysis gas chromatography. Sometimes microscopy is used only as a preliminary examination technique or to provide additional confirmatory information, but in other cases complete identifications are carried out. In some cases, identification may be impossible or very expensive by any other available technique. A few examples of applications of microscopical techniques are given below.

9.1 Identification of asbestos types

With the recognition of the lung-hardening and cancer-causing properties of respirable asbestos fibres came a need for a quick, cheap and simple method for the recognition of asbestos and identification of the type.

Identification by chemical analysis or X-ray diffraction would be time-consuming and sometimes inconclusive. The use of infra-red spectroscopy would involve careful sample preparation and possibly computer processing of spectra. Optical microscopy provides the quickest and simplest techniques for asbestos identification: examination of the optical properties of the different forms of asbestos[17] shows that observation of dispersion staining colours and sign of elongation are adequate. Julian and McCrone[18] showed that a selection of samples of asbestos could be classified into crocidolites, amosites, anthophyllites and chrysotiles by these means. Numerous asbestos samples have been identified in Materials Department, and the technique is now used routinely in Safety Section, Technical Facilities Department.

9.2 Investigations of contamination

Hot stage and dispersion staining microscopy suggested that white particulate contamination in an RAE laboratory was polystyrene; this was confirmed by pyrolysis-gas chromatography.

Dust from an air bearing was shown morphologically to contain oil-fired boiler soot, quartz, cotton, synthetic fibres, glass fibres and wood. Metal particles were picked out under the microscope with a needle and mounted on a 'brush' electrode[19,20] for emission spectrographic identification.

By a combination of small-scale emission spectroscopy, dispersion staining and a chemical test under the microscope, a very small sample of a contaminant found in an instrument mounted in an aircraft was identified as cadmium carbonate.

Microscopical examination showed that a deposit found in an optical encoder was in the form of small transparent spheres, many of which had shattered, causing local damage to a delicate surface. The melting point determined on the high-temperature hot stage and the refractive index indicated a soft glass. The contaminant was traced to a cleaning process.

9.3 Accurate refractive index determinations

During development on laser techniques it was required to know whether small differences existed between the refractive indices of individual glass cover slips. Using the single variation method at the wavelength of the laser, repeated determinations of refractive index were made on several cover slips so that the variations within and between individuals could be compared. It was shown that measurable differences occurred between individual cover slips.

Similar determinations have been made on vitreous silica in a space application.

9.4 Accident investigations

Together with emission spectroscopy, absorption spectrophotometry and X-ray diffraction, microscopy has been used to compare marks on wreckage with possible sources to study impacts during break-up of aircraft, *eg* behaviour on the hot stage provided evidence that a paint mark originated from another suspected aircraft. Hot stage behaviour and refractive index measurements were used together with pyrolysis-gas-liquid-chromatography to identify very small amounts of fibres found attached to the rotor of a crashed helicopter, aiding discovery of the cause of rotor fouling. Several microscopical techniques helped to show that a suspected sealant was not the material found embedded in a scratched metal surface from wreckage.

9.5 Examination of unknown materials

Melded-fibre materials of unknown composition have been examined by microscopy help identify the component materials and the method of manufacture. Hot stage micro has shown clearly when a fibre was coated with another material of lower melting point. The average thickness of such a coating can be measured and the positions of fibres of different materials shown by polarised light and dispersion staining techniques.

A number of materials from aircraft furnishings have been identified by microscopy when infra-red spectroscopy has produced inconclusive results. Morphological characteristics were used to distinguish cotton and jute, which both give the infra-red spectrum of cellulose. A carpet backing was shown by microscopy to consist of different synthetic fibres in each direction which could then be separated to give infra-red spectra of each without interference. Microscopical examination of a seat packing material showed a melded structure as described above.

Appendix A

SETTING UP THE 'PHOTOPLAN' ILLUMINATION

Fig 1 shows most of the controls referred to. See also instruction book[21].

A.1 Transmitted light

Switch on light.

Focus on a specimen with one of the lower power objectives.

Close transmitted light field iris.

Focus field iris with condenser racking knob.

Centre field iris image with condenser centring controls.

Open field iris to cover whole field.

Swing in Bertrand lens.

Swing transmitted light lamphouse diffuser out (down).

Open condenser aperture iris to cover an estimated $\frac{7}{10}$ of the back focal plane.

Focus lamp filament with transmitted light lamphouse focus knob.

Centre the filament image with transmitted light lamphouse centring knobs.

Swing Bertrand lens out.

Swing transmitted light lamphouse diffuser in.

A.2 Incident light

Always keep incident light lamphouse field iris fully open: the incident light illuminator has its own field iris (front lever).

Close illuminator field iris and check for reasonable focus and centration.

Open field iris to cover whole field.

Swing in Bertrand lens.

Swing incident light lamphouse diffuser out (down).

Open illuminator aperture iris (back lever) to fill an estimated $\frac{7}{10}$ of the back focal plane.

Focus filament with incident light lamphouse focus knob.

Centre filament image with incident light lamphouse centring knobs.

Swing Bertrand lens out.

Swing incident light lamphouse diffuser in.

Appendix B

PHOTOMICROGRAPHY

Fig 2 shows the apparatus.

B.1 Loading the camera

Full instructions for loading the 35 mm camera are given in the instruction book[22].

Load with Kodak Ektachrome ET 135-20 colour slide film: this can be processed by Printing Branch.

Store film in a refrigerator.

B.2 Setting up the camera

Replace the monocular head with the camera pillar (Fig 2). The head is held by a knurled, finger-tight screw just above the analyser. Open camera slide.

Insert the photocell of the exposure meter into the horizontal tube on the right of the pillar.

B.3 Taking photographs

Observe the specimen through the viewing eye-piece (lever at back in line with eye-piece).

Inner solid-line frame shows limits of field for 35mm camera. The frame graticule is focussed with the knurled ring of the eye-piece.

Swing eye-piece lever through 90 deg: light now passes to the photocell.

Check battery in exposure meter and take reading.

Required exposure = 2/meter reading seconds*.

Set the shutter speed to a value as close as possible to the calculated exposure.

Press the cable release.

Wind camera to next film position.

* When photographing samples between crossed polars, insert the quarter-wave compensator whilst making the light measurement.

Appendix C

REFRACTIVE INDEX DETERMINATION

C.1 Isotropic substances

Mount the wedge interference filter on the dovetail slide fitting below the condenser. Set to the required wavelength. Set up transmitted light illumination.

Place sample particles on a slide and cover with a number 1½, 16mm diameter cover slip.

Introduce a suitable Cargille refractive index liquid by capillary action between the slide and cover slip to immerse the sample.

Observe the Becke line (preferably at high magnification). Adjust condenser level and aperture to give optimum Becke effect.

On *raising* the focus, the Becke line moves towards the material of *higher* refractive index.

Repeat with different refractive index liquids until the particle is invisible. Then the refractive indices of solid and liquid are equal.

If the number of sample particles is limited, the refractive index measurement can be made by fixing the sample to the slide and changing the liquid on top of it, as follows.

Place the sample particle(s) on a clean slide, preferably in the laminar-flow clean bench, and add one small drop of collodion solution. Allow to dry to a flexible film in which the sample particles are embedded.

Under the microscope, at low magnification, cut out a section of the collodion film containing one particle, using a fine tungsten needle.

With the needle, transfer the sample in collodion to another slide. Add one drop of amyl acetate: the collodion dissolves and spreads out to a very thin film which still holds the particle to the slide but does not interfere in refractive index measurements.

Place a cover slip over the sample and immerse and observe Becke line as before.

To change the liquid, place a small piece of filter paper with its torn edge against one end of the cover glass and introduce the new liquid at the other.

Repeat as necessary until the matching liquid is found.

C.2 Anisotropic substances

Put the polariser in place with its vibration direction lying left to right.

For well-defined crystals or fibres, the appropriate direction can be aligned with that of the polariser and measurements made as for isotropic substances.

In the case of irregular fragments, the maximum and minimum refractive indices (*ie* ε and ω for uniaxial substances or α and γ for biaxial substances) can sometimes be estimated by the following tedious procedure.

Using crossed polars, find an extinction position of one of the fragments in a refractive index liquid.

Make an estimate of the refractive index from the relief: refractive index differences of 0.02, 0.05, 0.1 and 0.2 give low, moderate, high and very high relief respectively.

Rotate the sample through 90 deg to another extinction position and make another estimate on the same fragment.

Repeat the procedure for a number of fragments and find the maximum refractive index difference for a fragment.

Now mount some fragments in a liquid of approximately the minimum found above.

Once again, for a number of fragments, find two refractive indices by estimation.

Repeat in a liquid approximately to the higher refractive index.

NB The method is uncertain, time-consuming and very seldom worthwhile.

Appendix D

DISPERSION STAINING

D.1 Illumination

Mount sample in suitable refractive index liquid (very close to sample in refractive index).

Using the 10X dispersion staining objective with the stops out, focus on sample.

Swing in the Bertrand lens and central stop in objective.

Open condenser iris slightly and centre condenser with adjusting screws.

Close condenser iris to minimum and swing out Bertrand lens.

Lower condenser until best dark field and dispersion-staining colours appear (near lowest position of condenser).

D.2 Isotropic substances

Estimate wavelength of dispersion staining colours (central and annular stops) from charts or, with annular stop in, adjust wedge interference filter until particle 'disappears'.

D.3 Uniaxial substances

Insert polariser.

If possible, find a particle whose dispersion staining colour does not change on rotation of the stage (*ie* ω seen in all directions). Otherwise, find the *common colour*, *ie* the colour which appears for every particle at one of its pairs of extinction positions.

Use the wedge interference filter to measure the common colour. The common colour corresponds to ω.

If the common colour is nearer the red end of the spectrum ('higher'), with the annular stop, then the colours at the other pair of extinction positions, then $\omega < \varepsilon$. If it is nearer the blue end ('lower'), then $\omega > \varepsilon$.

If sufficient particles are present, find a particle which gives the dispersion staining colour spectrally farthest from the common colour at its other pair of extinction positions. Assuming a large number of particles, this should be ε. Measure the colour with the wedge interference filter.

D.4 Biaxial substances

No common colour is displayed.

Insert polariser.

Select a particle and measure the dispersion staining colour at opposite extinction positions. Record as 'high' and 'low' colours.

Repeat measurements on a number of different particles.

The lowest wavelength in the 'low' set represents α and the highest in the 'high' set represents γ . β cannot be measured, except in rare cases in which β happens to be displayed in all directions and the particle appears isotropic.

Appendix E

DENSITY DETERMINATION

Melting-point glass capillaries should be cleaned batchwise with Decon 90, rinsed with water and dried with reagent grade acetone.

Lay a clean capillary in the horizontal groove on the wooden block (Fig 9).

From a hypodermic syringe introduce sufficient di-iodomethane to fill half the length of the capillary.

Similarly, from the same end of the capillary, fill the remainder of the capillary with xylene, taking care to avoid a bubble between the liquids.

Plug the high-density end of the capillary with 'Blu-tack'.

Stand the capillary vertically in a hole in the block.

From the point of a needle, introduce particles of the sample and at least three suitable density standards (Table I) by touching the particles onto the liquid surface. Top up the xylene from the syringe if necessary.

Holding the top of the capillary lightly between finger and thumb, gently top the capillary, rotating it at the same time. Allow to stand vertically for about 5 min, or longer if the particles are very small.

Place the capillary into the immersion cell containing cedarwood oil.

View with the travelling vernier microscope.

Measure the positions of the sample and standard particles with the vernier scale.

Plot the positions of the standards against their known densities and, from the position of the sample, find its density to one decimal place.

If it is required to collect fractions from the density gradient, remove the capillary from the immersion cell, wipe off excess cedarwood oil and lay it carefully back in the horizontal groove.

Carefully remove the 'Blu-tack' plug with a fine needle.

By slightly tilting the block whilst the capillary is held in place on it, touch the open end quickly onto different areas in turn around the edge of a membrane filter. A watchmaker's eye-glass or magnifying viewer may be necessary.

Particles may be examined whilst on the membrane filter or transferred with a fine needle to a slide.

A membrane filter (Millipore HAWP) may be made transparent by immersing it in amyl salicylate (refractive index 1.510).

Appendix F

IDENTIFICATION OF ASBESTOS TYPES

F.1 Sign of elongation

Insert polariser and analyser.

Set first-order-red compensator with its + direction along the length of the slide. Insert in tube.

Mount fibres in Cargille immersion liquid, refractive index 1.690.

If the fibres have blue polarisation colours when lying bottom left to top right of field and orange polarisation colours when lying bottom right to top left of field, they are optically positive.

If the opposite is found, the fibres are optically negative. Crocidolite is the only optically negative form of asbestos found.

F.2 Dispersion staining

Test fibres for dispersion staining colours in liquids with refractive indices 1.690, 1.610 and 1.550.

Crocidolite and amosite both give dispersion staining colours in liquid of refractive index 1.690 but not in the others.

Anthophyllite gives dispersion staining colours in liquids of refractive index 1.610 but not in the others.

Chrysotile gives dispersion staining colours in liquid of refractive index 1.550 but not in the others.

To distinguish between crocidolite and amosite, the sign of elongation is used. The sign of elongation can be confirmed from the dispersion staining colours observed with the fibres lying vertically and horizontally in the field. Crocidolite gives a *lower* dispersion staining colour for vertical fibres than for horizontal. Amosite gives a *higher* colour for vertical fibres.

F.3 Removal of interfering particles

In many of their commonly used forms, asbestos fibres are bound together with inorganic cements. Particles of these binders often seriously affect the dispersion staining colours of the asbestos itself and need to be removed. This can usually be accomplished by treating the material with acetic acid, rinsing with water and drying.

Table 1

DENSITY STANDARDS

Substance	Density
Diethylamine hydrochloride	1.048
Glycine	1.161
Ethyl urea	1.213
Fuchsin	1.22
Ammonium magnesium chloride, $NH_4 Mg Cl_3.6H_2O$	1.456
Ammonium alum	1.64
Potassium alum	1.76
Ammonium dihydrogen phosphate	1.803
Ammonium borofluoride	1.851
Ammonium perchlorate	1.95
Potassium chloride	1.988
Potassium nitrate	2.11
Ammonium dichromate	2.15
Sodium chloride	2.163
Sodium nitrate	2.257
Gypsum	2.32
Potassium perchlorate	2.524
Quartz	2.653
Calcite	2.711
Potassium bromide	2.75
Dolomite	2.873
Barium chloride	3.097
Calcium fluoride	3.18
Barium nitrate	3.244

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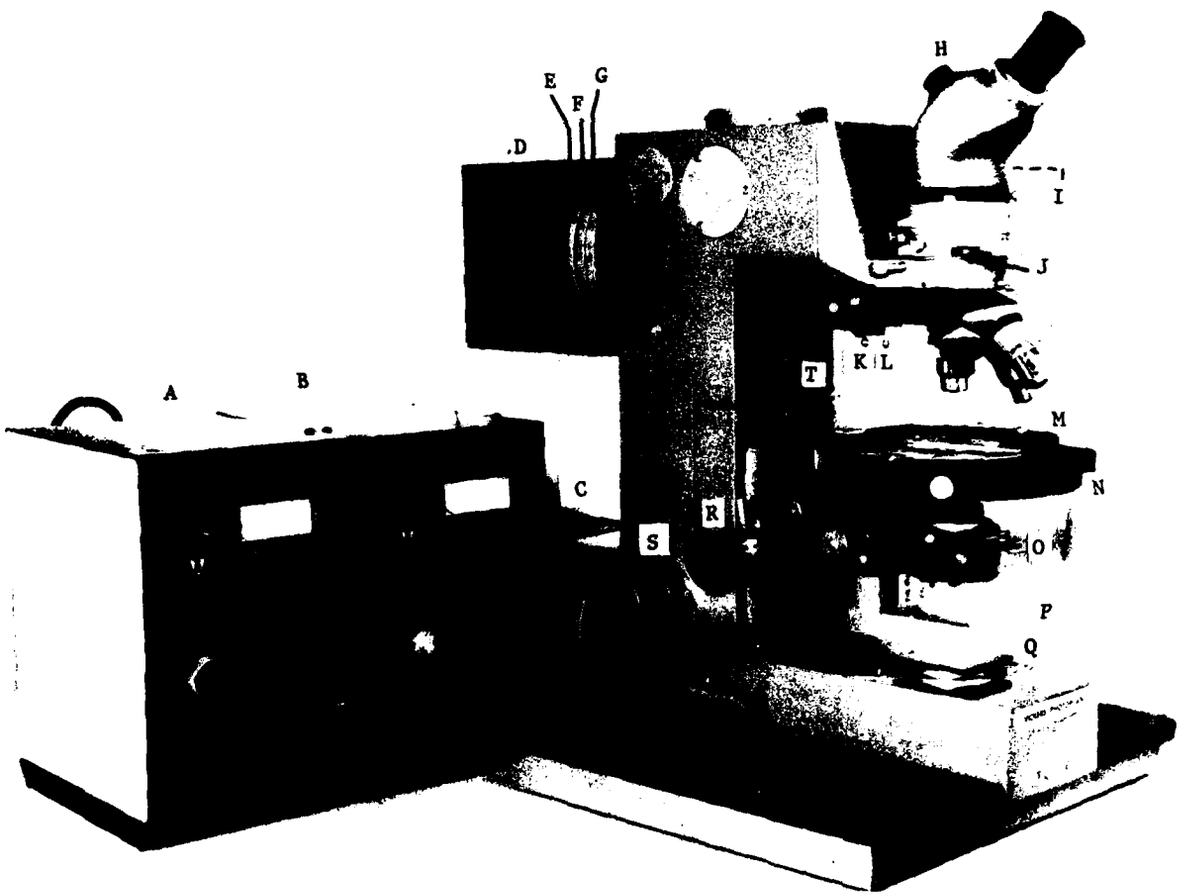
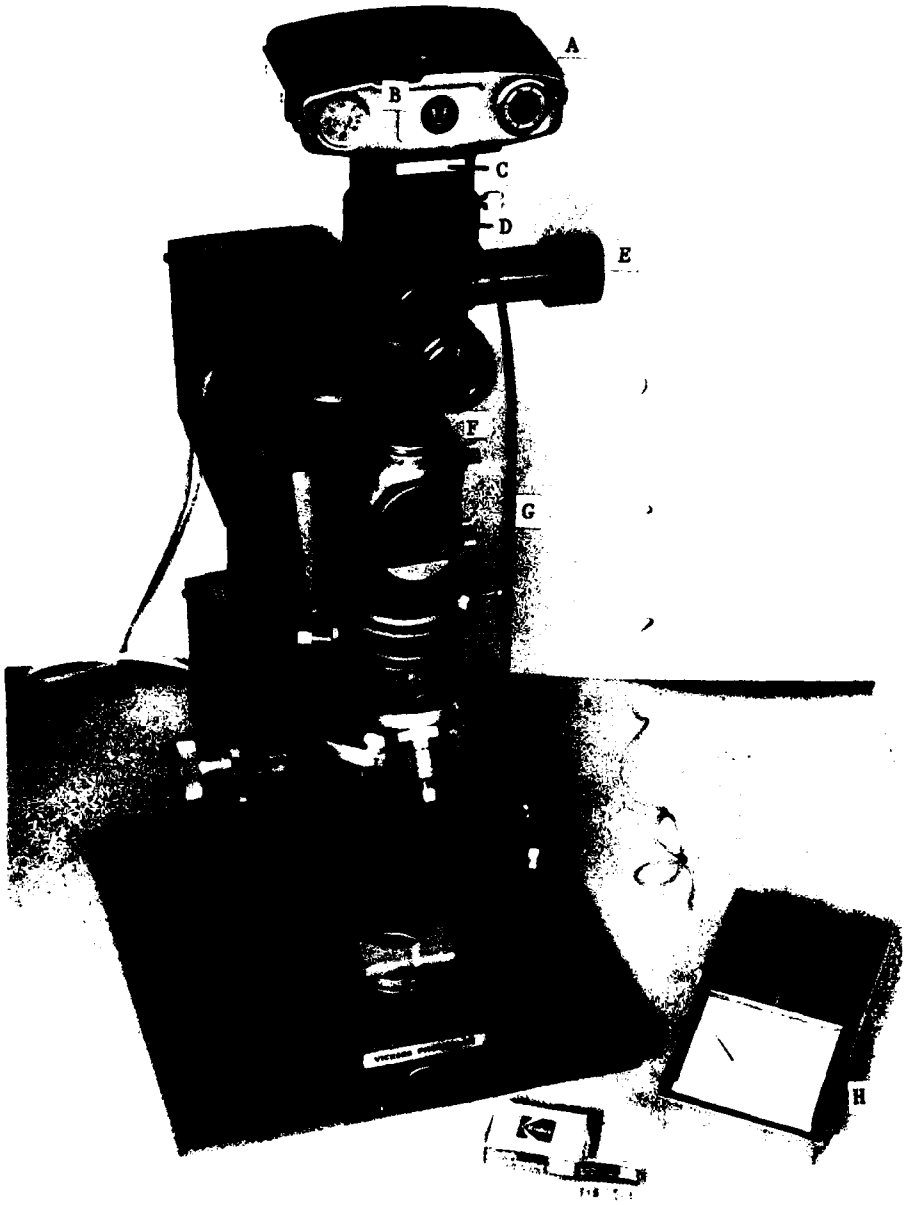


A	Transmitted light intensity control
B	Incident light intensity control
C	Transmitted light lamphouse
D	Incident light lamphouse
E	Field iris
F,G	Filters
H	Bertrand lens control
I	Analyser in/out
J	Compensators
K	Incident light aperture iris
L	Incident light field iris
M	Detachable vernier mechanical stage
N	Rotating stage
O	Condenser
P	Slide for attaching wedge interference filter
Q	Transmitted light polariser
R	Coarse focus
S	Fine focus
T	Incident light polariser

Fig 1 Basic microscope

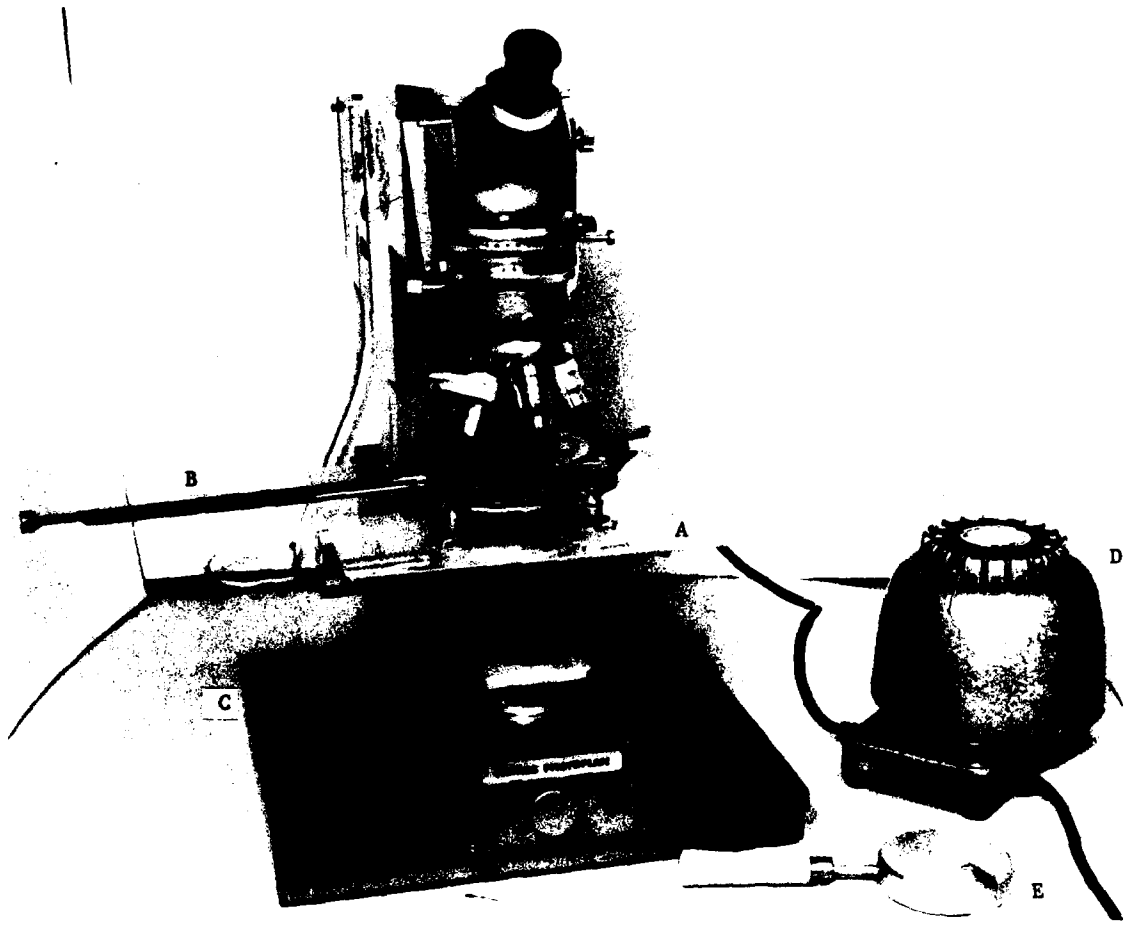
Fig 2



A	Camera body
B	Film wind lever
C	Camera slide
D	Shutter speed control
E	Photocell
F	Viewing eyepiece
G	Cable release
H	Exposure meter

Fig 2 Apparatus for photomicrography

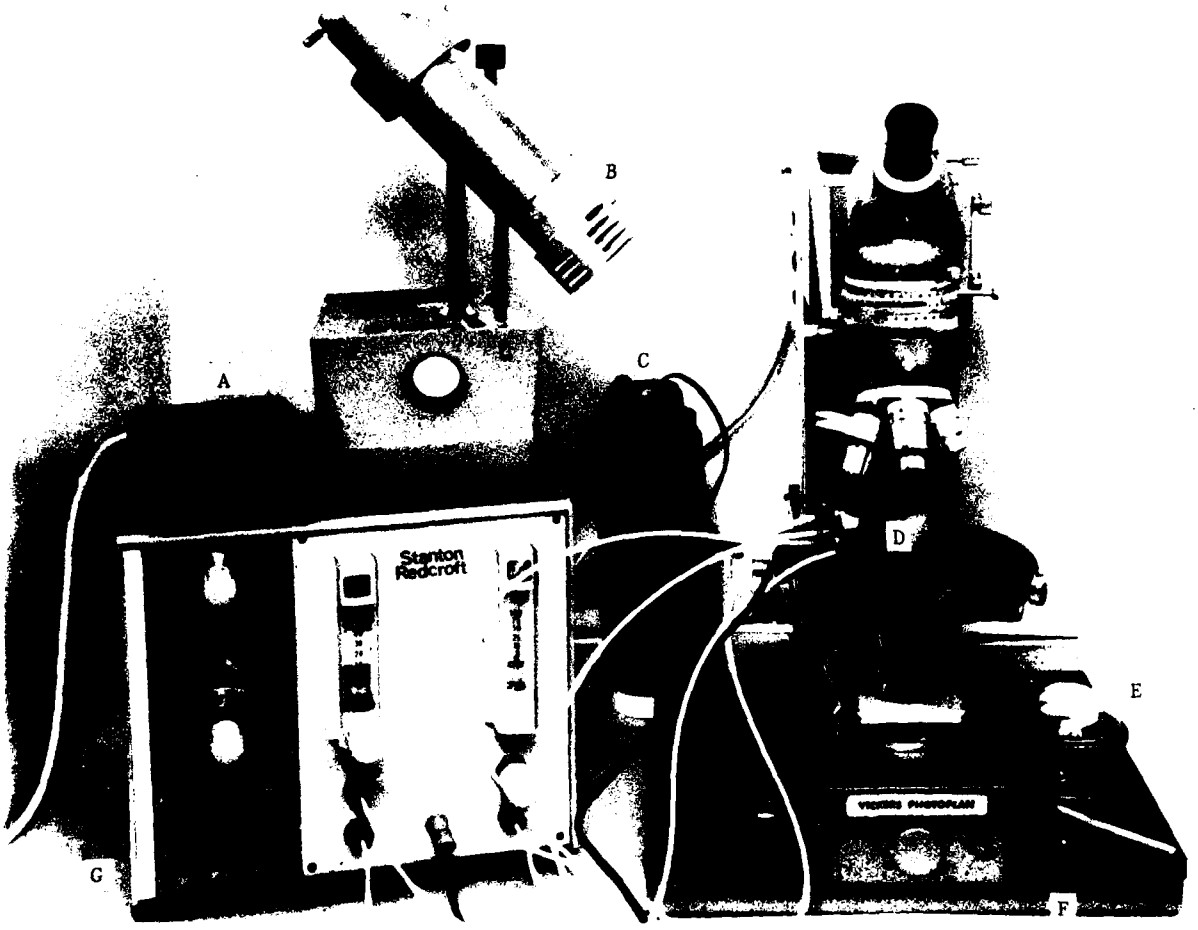
TM Mat 397 C17620



A Mounting plate
B Thermometer for one range, in support
C Thermometer for other range
D Temperature control unit
E Cooling block

Fig 3 Kofler hot stage

Fig 4



A Digital voltmeter
B Lamp providing oblique incident light
C Thermocouple cold junction in flaked ice
D Hot stage
E Micro desiccator for pans
F Pan
G Temperature programmer/flow control unit

Fig 4 High-temperature hot stage

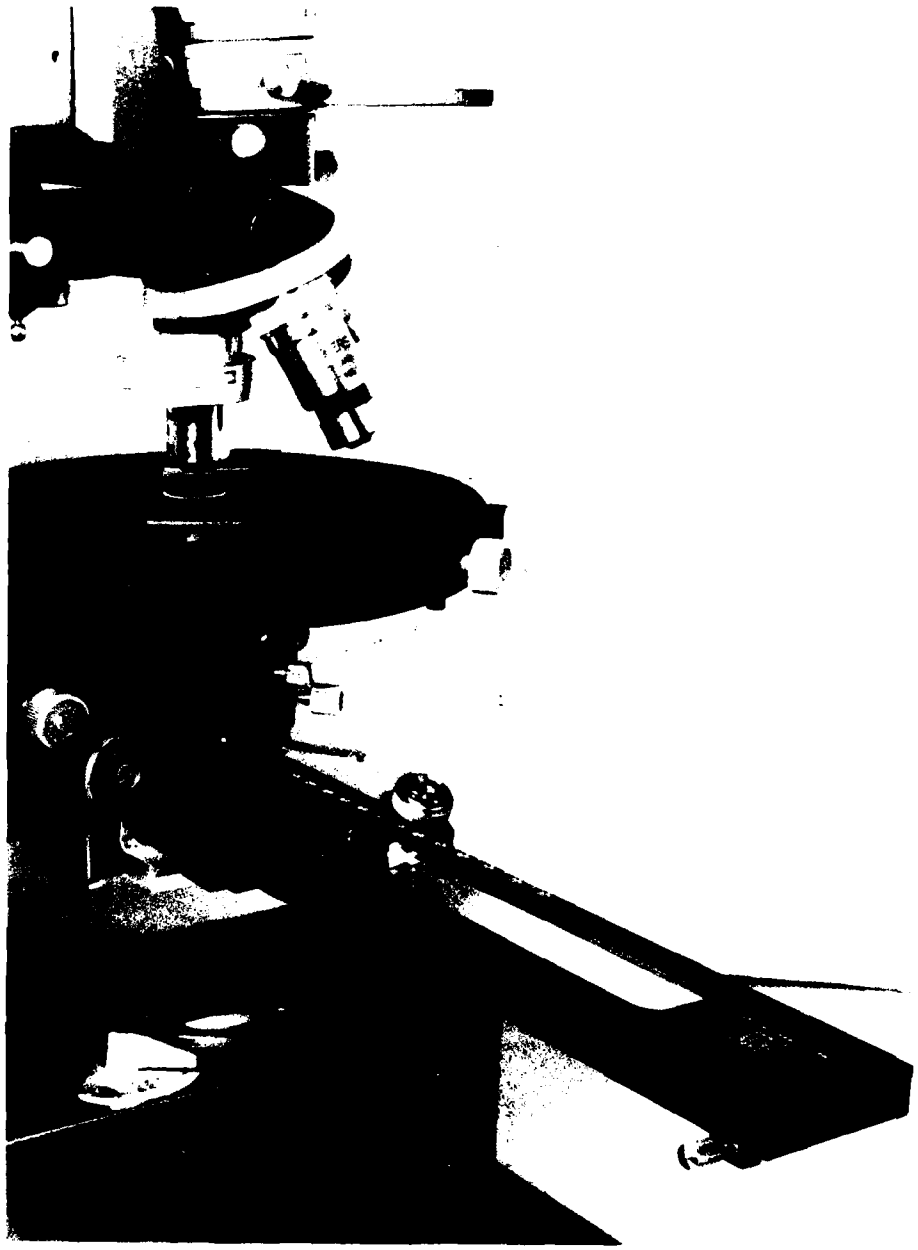


Fig 5 Wedge interference filter

Fig 6

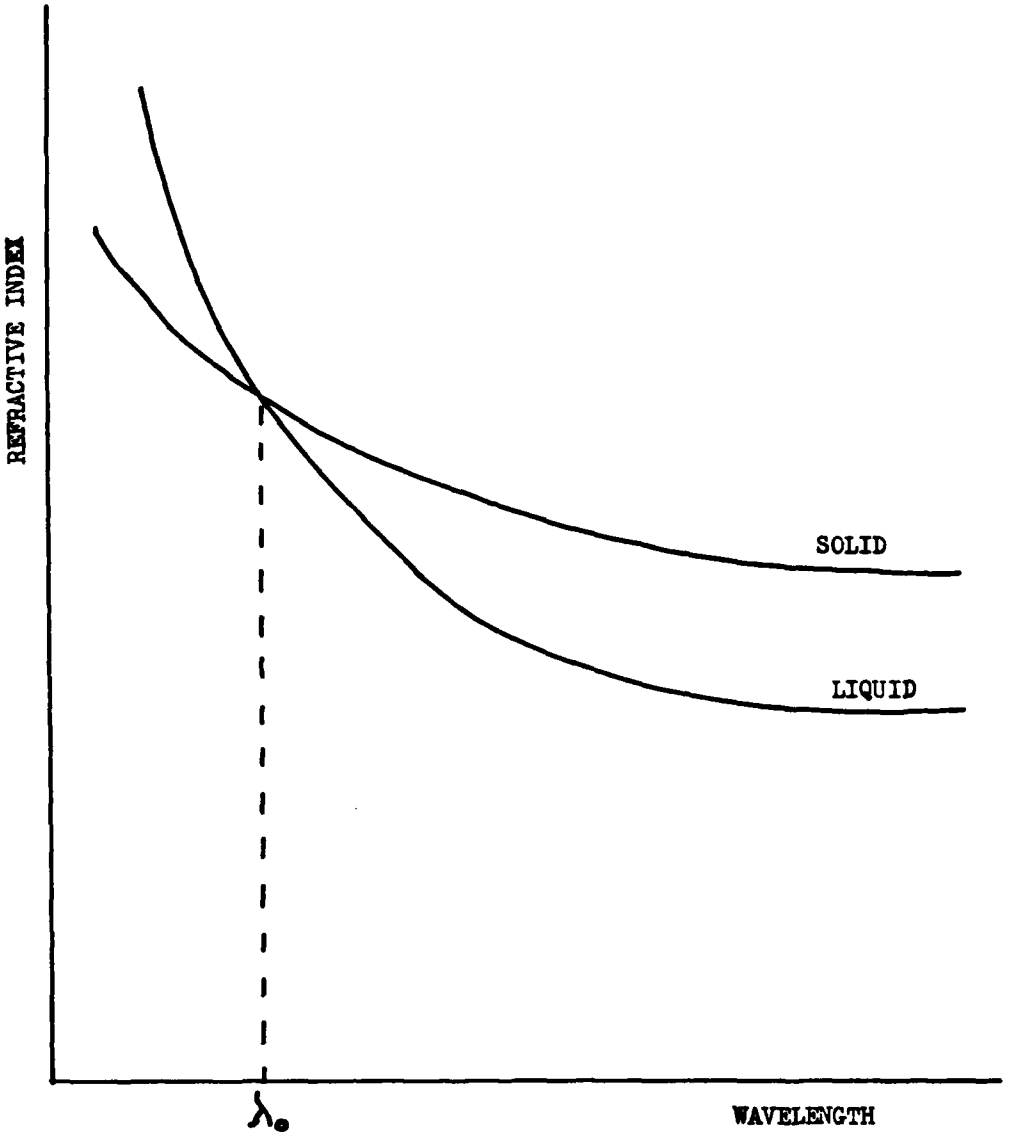


Fig 6 Dispersions of solid and liquid

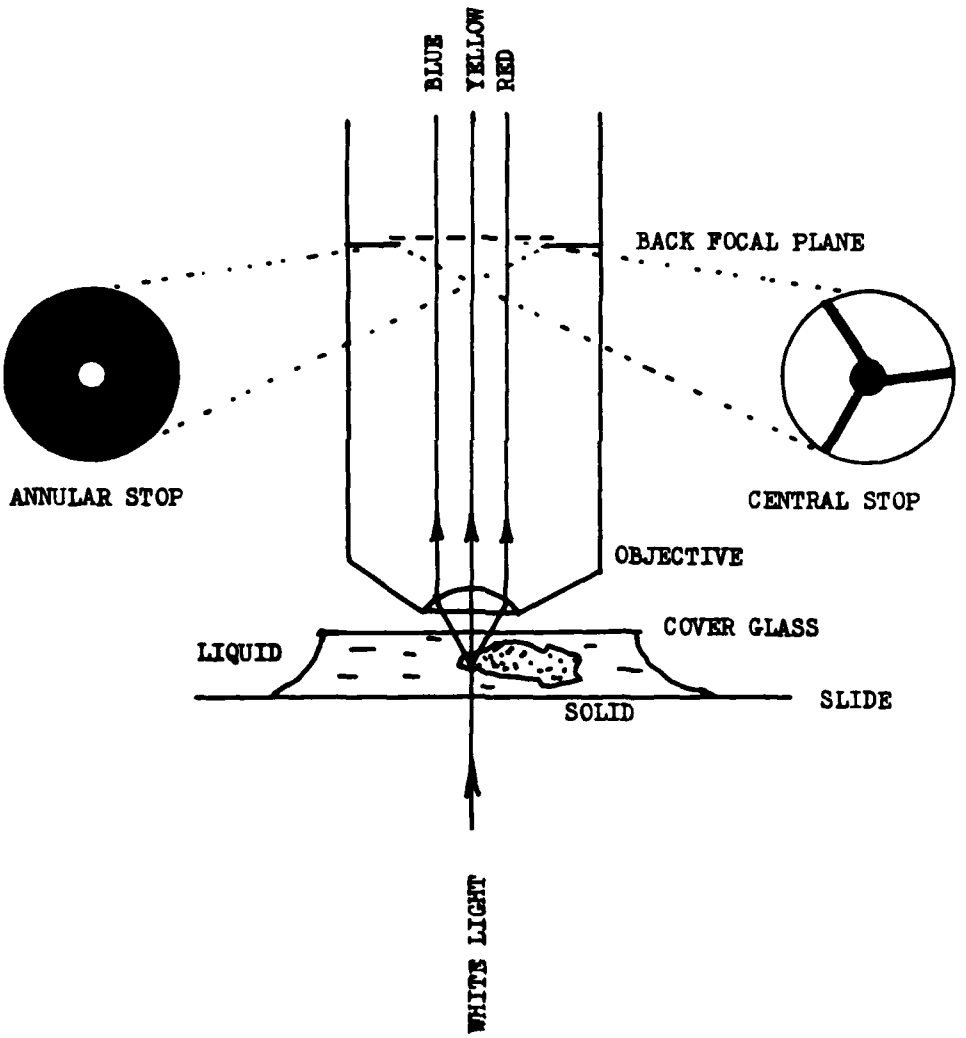


Fig 7 Optics for dispersion staining

Fig 8

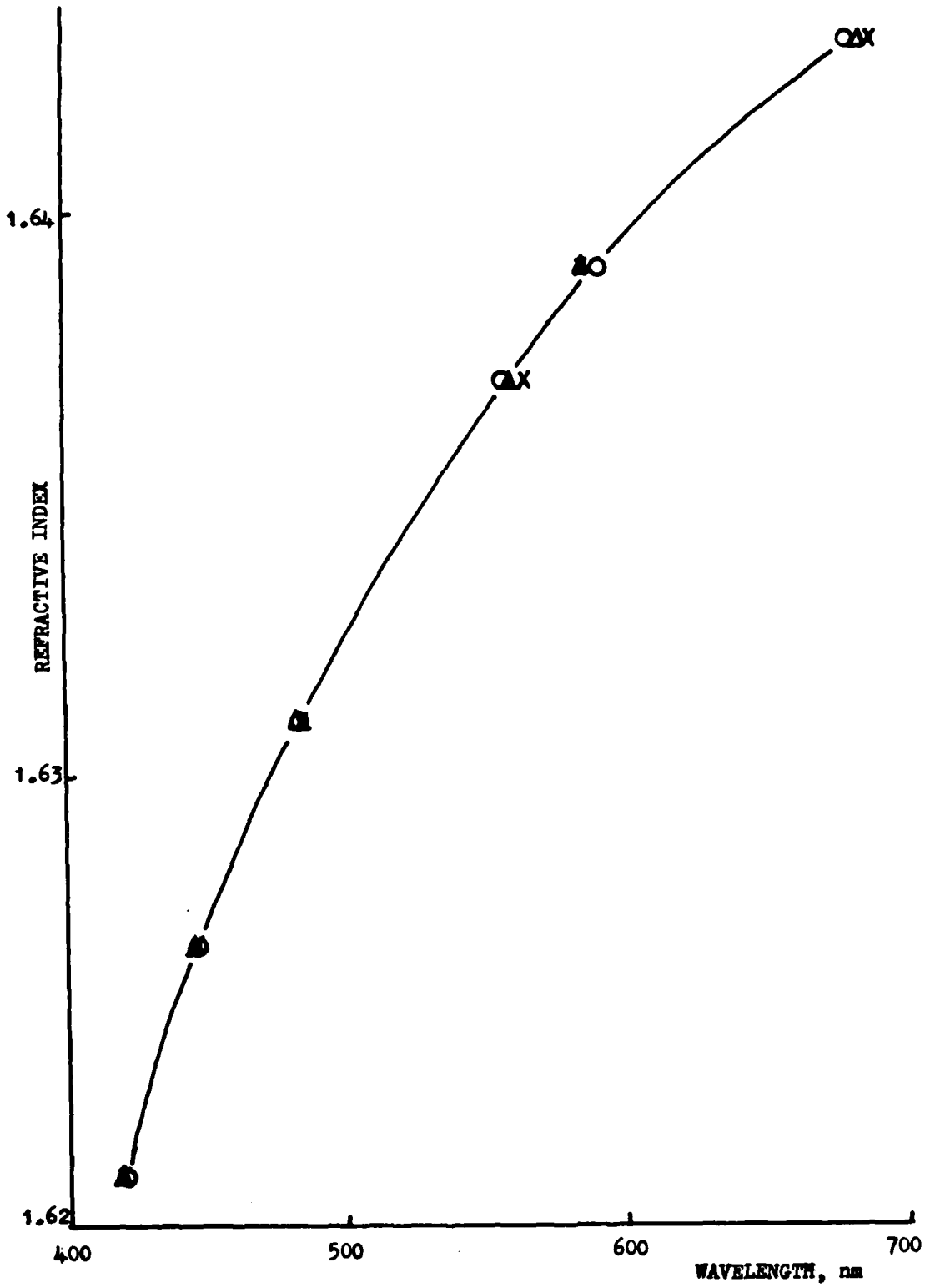
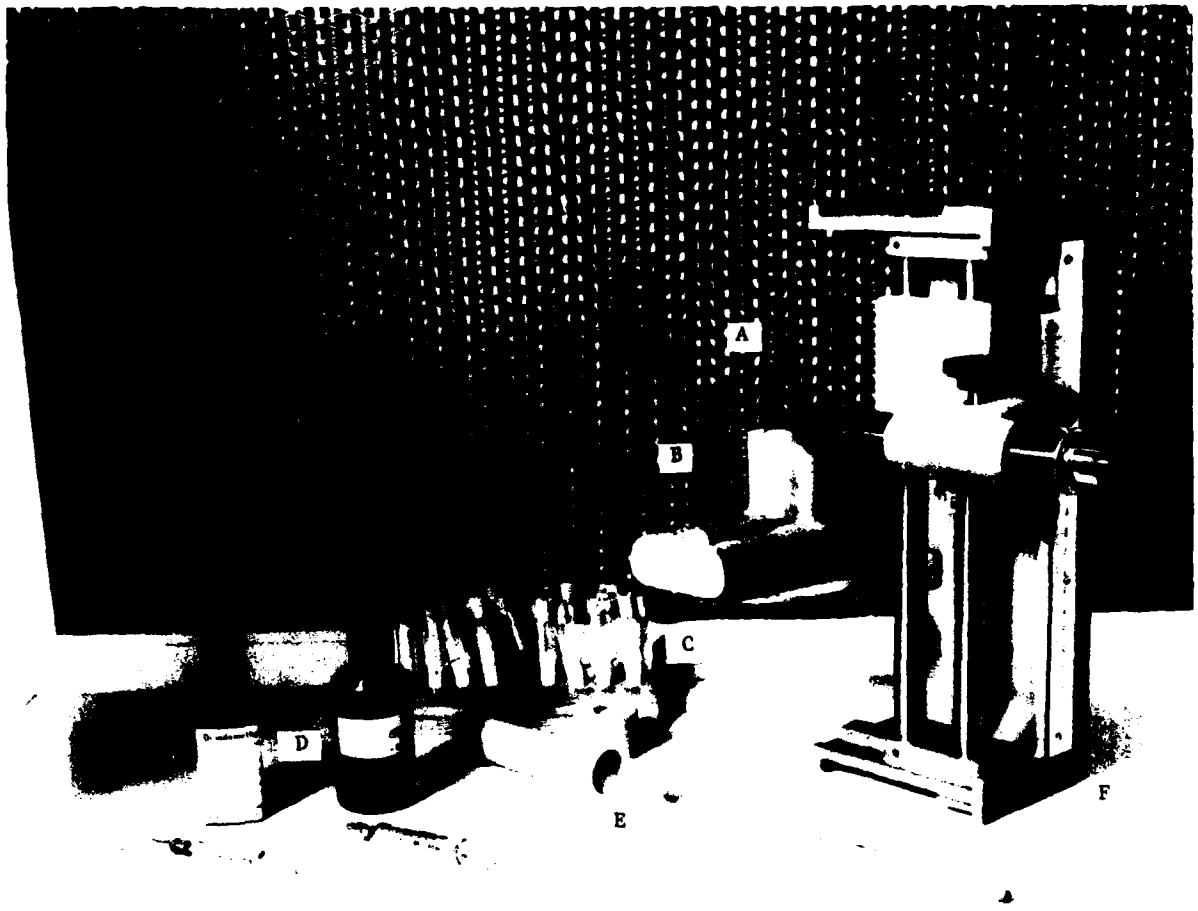


Fig 8 Dispersion staining curve for ammonium chloride



A Capillary tube containing density gradient
B Immersion cell
C Density standards
D Liquids for forming density gradient
E Melting-point capillary tubes
F Travelling microscope

Fig 9 Apparatus for density determination

Fig 10

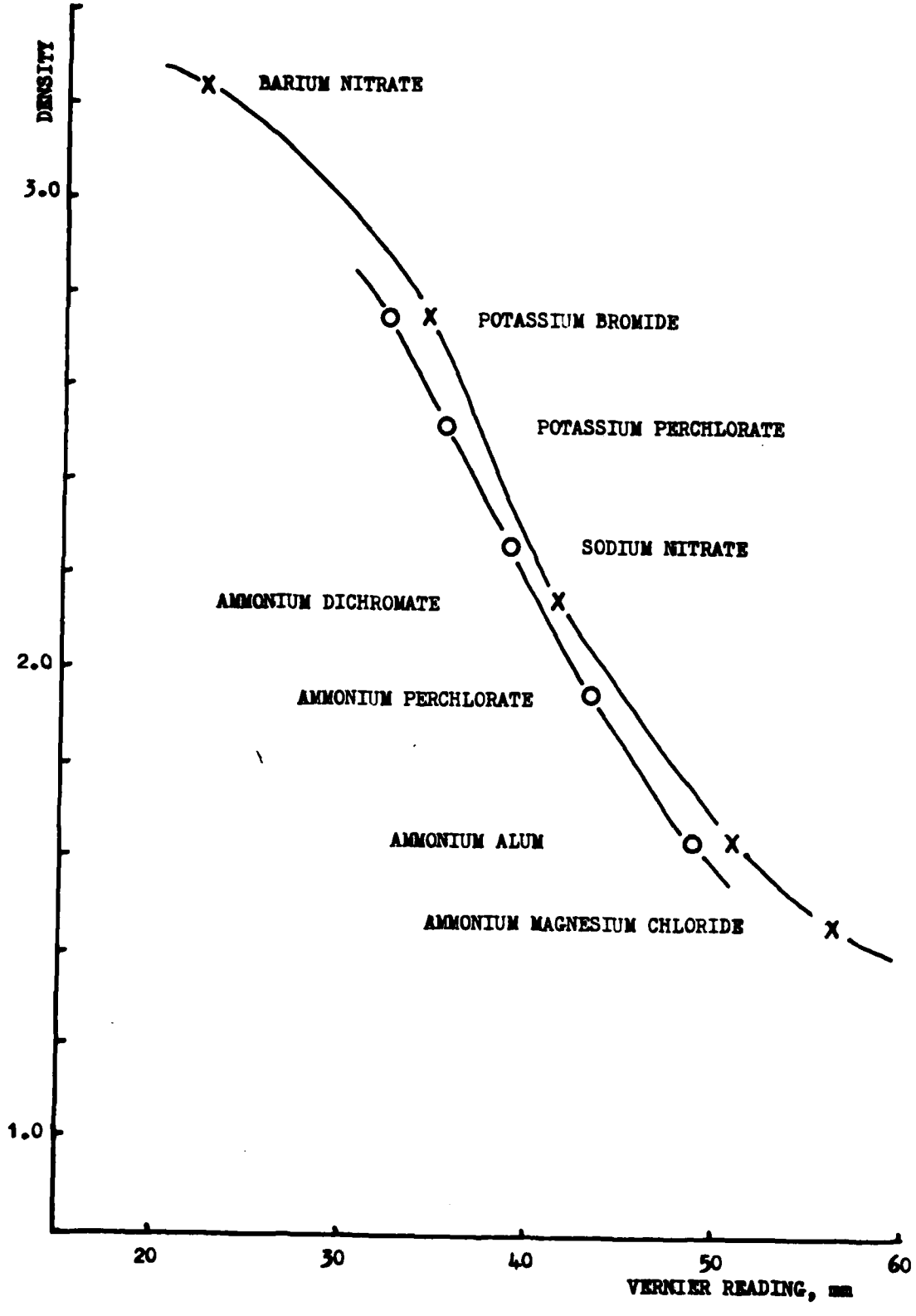
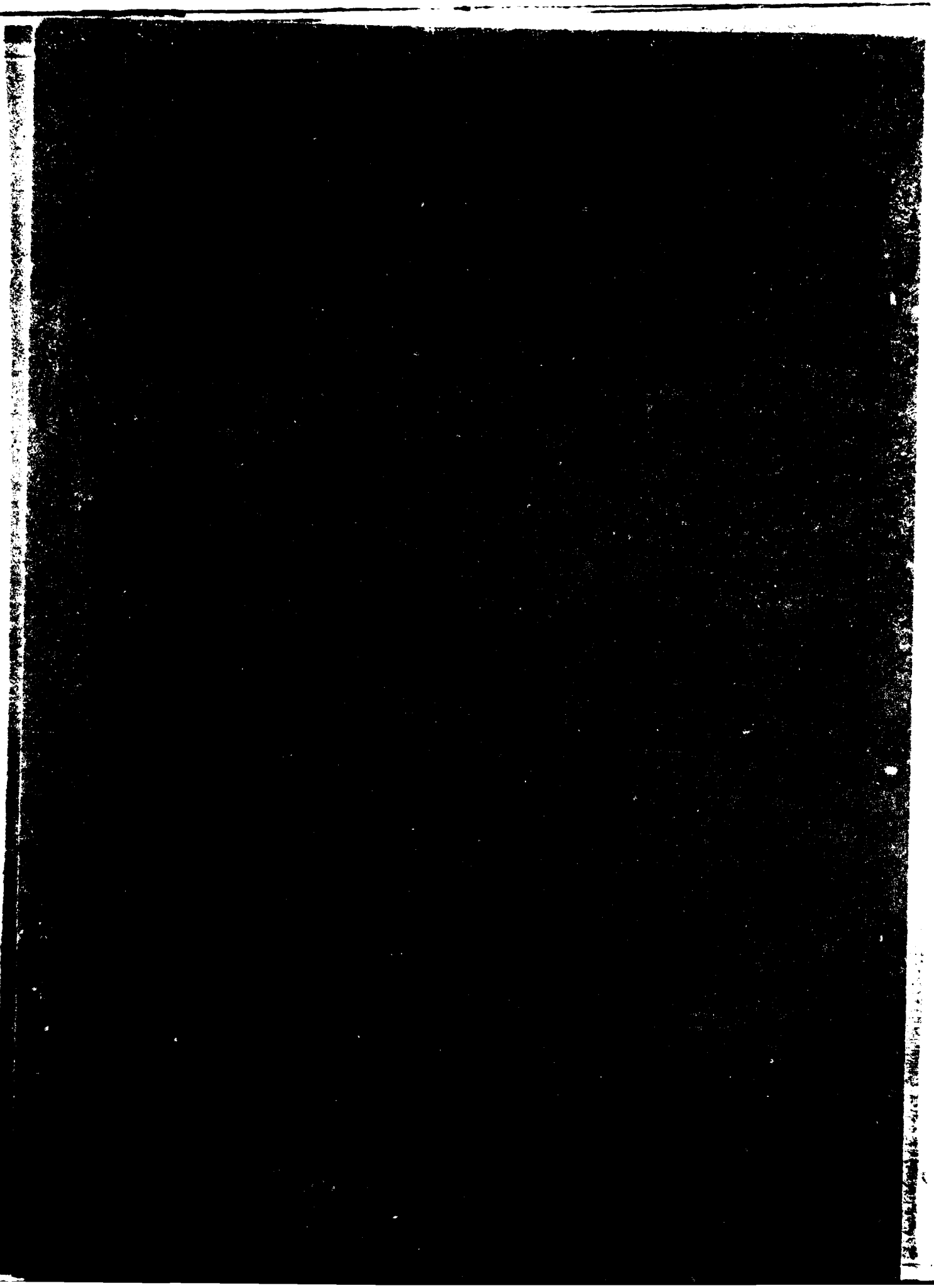


Fig 10 Typical density gradient calibrations

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